

CLAIMS

We claim,

1. A method for identifying a target for antibacterial agents, comprising determining the bacterial target of a product of a bacteriophage 44AHJD open reading frame selected from the group consisting of open reading frames 12 and 25.
2. The method of claim 1, wherein said determining comprises identifying at least one bacterial protein which binds to said product or a fragment thereof.
3. The method of claim 2, wherein said binding is determined using affinity chromatography on a solid matrix.
4. The method of claim 1, wherein said determining comprises identifying at least one protein:protein interaction using a genetic screen.
5. The method of claim 4, wherein said genetic screen is a yeast two-hybrid screen.
6. The method of claim 1, wherein said determining comprises a co-immunoprecipitation assay or a protein-protein crosslinking assay.
7. The method of claim 1, wherein said determining comprises identifying a mutated bacterial coding sequence which protects a bacterium from said bacteriophage 44AHJD open reading frame product.
8. The method of claim 1, wherein said determining comprises identifying a bacterial coding sequence which protects a bacterium against said product when expressed at high levels in said bacterium.
9. The method of claim 1, wherein said determining further comprises identifying a bacterial nucleic acid sequence encoding a polypeptide target of said product of bacteriophage 44AHJD open reading frame.
10. The method of claim 9, wherein said nucleic acid sequence is identified by determining at least a fragment of the amino acid sequence of a bacterial protein target, and identifying a bacterial nucleic acid sequence which encodes said fragment.
11. The method of claim 1, wherein said bacterial target is from an animal pathogen.
12. The method of claim 11, wherein said bacterial target is a gene homologous to a gene from an animal pathogen.
13. The method of claim 11, wherein said pathogen is a human pathogen.

14. The method of claim 1, wherein said bacterial target is from a plant pathogen.
15. The method of claim 1, wherein said bacterial target is a gene homologous to a gene from a plant pathogen.
16. The method of claim 1, further comprising determining the cellular or biochemical function or both of said product of bacteriophage 44AHJD open reading frame.
17. The method of claim 1, wherein said identifying the bacterial target comprises identifying a phage-specific site of action.
18. An isolated, purified, or enriched nucleic acid sequence at least 15 nucleotides in length, wherein said sequence corresponds to at least a portion of a bacteriophage 44AHJD open reading frame 12 or 25 sequence.
19. The nucleic acid sequence of claim 18, wherein said sequence comprises at least 50 nucleotides.
20. The nucleic acid sequence of claim 18, wherein said nucleic acid sequence corresponds to a fragment of said bacteriophage 44AHJD open reading frame 12 or 25 sequence.
21. The nucleic acid sequence of claim 20, wherein said nucleic acid sequence encodes a polypeptide which provides a bacteria-inhibiting function.
22. The nucleic acid sequence of claim 21, wherein said nucleic acid sequence is transcriptionally linked with regulatory sequences enabling induction of expression of said sequence.
23. An isolated, purified, or enriched polypeptide comprising at least a fragment of a protein encoded by *Staphylococcus aureus* bacteriophage 44AHJD open reading frame 12 or 25, wherein said portion is at least 5 amino acid residues in length.
24. The polypeptide of claim 24, wherein said polypeptide comprises a fragment at least 10 amino acid residues in length of a said polypeptide normally encoded by said bacteriophage.
25. A recombinant vector comprising a nucleic acid sequence at least 24 nucleotides in length corresponding to a portion of bacteriophage 44AHJD open reading frame 12 or 25.
26. The vector of claim 25, wherein said vector is an expression vector.
27. The vector of claim 26, wherein expression of said ORF is inducible.

28. A recombinant cell comprising a vector, wherein said vector comprises a nucleic acid sequence at least 24 nucleotides in length, corresponding to at least a fragment of bacteriophage 44AHJD open reading frame 12 or 25.

29. The cell of claim 28, wherein said vector is an expression vector and expression of said ORF is inducible.

30. A method for identifying an antibacterial agent, comprising identifying an active fragment of a product of a bacteriophage-inhibiting ORF of a bacteriophage.

31. The method of claim 30, further comprising constructing a synthetic peptidomimetic molecule, wherein the structure of said molecule corresponds to the structure of said active fragment.

32. A method for identifying a compound active on a bacterial target protein of a bacteriophage 44AHJD open reading frame 12 or 25 product, comprising the step of contacting said bacterial target protein with a test compound; and determining whether said compound binds to or reduces the level of activity of said target protein, wherein binding of said compound with said target protein or a reduction of the level of activity of said protein is indicative that said compound is active on said target and wherein said target is uncharacterized.

33. The method of claim 32, wherein said contacting is carried out in vitro.

34. The method of claim 32, wherein said contacting is carried out in vivo in a cell.

35. The method of claim 32, wherein said compound is a small molecule.

36. The method of claim 32, wherein said compound is a peptidomimetic compound.

37. The method of claim 32, wherein said compound is a fragment of a bacteriophage inhibitor protein.

38. The method of claim 32, further comprising determining the site of action of said compound on said target protein.

39. A method of screening for potential antibacterial agents, comprising the step of determining whether any of a plurality of compounds is active on a target of a bacteriophage 44AHJD open reading frame 12 or 25 product,

wherein said target is naturally produced by a pathogenic bacterium. [one step method claim]

40. The method of claim 39, wherein said ~~plurality~~⁶² of compounds are small molecules.

41. A method for inhibiting a bacterium, comprising the step of;

5 contacting said bacterium with a compound active on a target of a bacteriophage 44AHJD open reading frame 12 or 25 product, wherein said target or target site is uncharacterized.

42. The method of claim 41, wherein said compound is said protein or an active fragment thereof.

10 43. The method of claim 41, wherein said compound is a structural mimetic of said protein.

44. The method of claim 41, wherein said compound is a small molecule.

45. The method of claim 41, wherein said contacting is performed in vitro.

46. The method of claim 41, wherein said contacting is performed in vivo in an animal.

15 47. The method of claim 41, wherein said animal is a human.

48. The method of claim 41, wherein said contacting is carried out in vivo in a plant.

49. The method of claim 41, wherein said bacterium is pathogenic.

50. A method for treating a bacterial infection in an animal suffering from an infection, comprising administering to said animal a therapeutically effective amount of compound
20 active on a target of a bacteriophage 44AHJD open reading frame 12 or 25 product in a bacterium involved in said infection.

wherein said target is an uncharacterized target or the compound is active at an uncharacterized target site.

51. The method of claim 50, wherein said compound is a small molecule.

25 52. The method of claim 50, wherein said compound is a peptidomimetic compound.

53. The method of claim 50, wherein said compound is a fragment of a bacteriophage inhibitor protein.

54. The method of claim 50, wherein said animal is a mammal.

55. The method of claim 54, wherein said mammal is a human.

56. A method for prophylactically treating an animal at risk of an infection, comprising administering to said animal a prophylactically effective amount of a compound active on a target of a bacteriophage 44AHJD open reading frame 12 or 25 product, wherein said target is an uncharacterized target or the site of action of said compound is an uncharacterized target site.

57. The method of claim 56, wherein said compound is a small molecule.

58. The method of claim 56, wherein said compound is a peptidomimetic compound.

59. The method of claim 56, wherein said compound is a fragment of a bacteriophage inhibitor protein.

60. The method of claim 56, wherein said animal is a mammal.

61. The method of claim 60, wherein said mammal is a human.

62. An antibacterial agent active on a target of a bacteriophage 44AHJD open reading frame 12 or 25 product, wherein said target is an uncharacterized target or said agent is active at a phage-specific site on said target.

63. The agent of claim 62, wherein said agent is a peptidomimetic of a bacteriophage inhibitor polypeptide.

64. The agent of claim 62, wherein said agent is a small molecule.

65. The agent of claim 62, wherein said agent is a fragment of a bacteriophage inhibitor polypeptide.

66. The agent of claim 62, wherein said agent is active at a phage-specific site on said target.

67. A method of making an antibacterial agent, comprising the steps of: identifying a target of a bacteriophage 44AHJD open reading frame 12 or 25 product; screening a plurality of test compounds to identify a compound active on said target; and synthesizing said compound in an amount sufficient to provide a therapeutic effect when administered to an organism infected by a bacterium naturally producing said target.

68. The method of claim 67, wherein said compound is a small molecule.

69. The method of claim 67, wherein said compound is a peptidomimetic compound.

70. The method of claim 67, wherein said compound is a fragment or derivative of a bacteriophage 44AHJD open reading frame product.

71. An antibody which binds a protein encoded by an open reading frame from *Staphylococcus aureus* bacteriophage 44AHJD.

72. The antibody of claim 71, wherein said antibody binds a protein which corresponds to a protein encoded by an open reading frame from *Staphylococcus aureus* bacteriophage 44AHJD.

73. A method for detecting a phage protein comprising the steps of, contacting said phage with an antibody, wherein said antibody binds a protein encoded by an open reading frame from *Staphylococcus aureus* bacteriophage 44AHJD.

74. A method for detecting a virus comprising the steps of, contacting said virus with an antibody, wherein said antibody binds a protein encoded by an open reading frame from *Staphylococcus aureus* bacteriophage 44AHJD.

75. The method of claim 74, wherein said virus is pathogenic to a mammal.

76. The method of claim 75, wherein said mammal is a human.

77. A method for determining the cellular and/or biochemical function of a bacterial target of a bacteriophage 44AHJD open reading frame product comprising; contacting said bacterial target protein with a test compound; determining whether said compound binds to or reduces the level of activity of said target protein, and identifying homologous polypeptides and/or nucleic acid molecules to said target protein having known functions, wherein binding of said compound with said target protein or a reduction of the level of activity of said protein is indicative that said compound is active on said target and wherein said target is uncharacterized.

78. The method of claim 77, wherein said contacting is carried out in vitro.

79. The method of claim 77, wherein said contacting is carried out in vivo in a cell.

80. The method of claim 77, wherein said compound is selected from the group consisting of a small molecule, a peptidomimetic compound, or a fragment or derivative of a bacteriophage inhibitor protein.

81. A method of screening for compounds that inhibit an *S. aureus* dnaN product, comprising

contacting said dnaN product with a bacteriophage 44AHJD ORF25 product or a
5 fragment thereof and at least one test compound, and

determining whether any of said test compounds reduces the interaction between said dnaN product and said ORF25 product, wherein a reduction in said interaction is indicative that said test compound inhibits said dnaN product.

10 82. The method of claim 81, wherein said dnaN product has the amino acid sequence of SEQ ID NO:2.

83. The method of claim 81, wherein said determining comprises measuring the interaction between dnaN and ORF 25 product, wherein dnaN or ORF25 product is directly
15 labeled.

84. The method of claim 83, wherein said dnaN product comprises an active portion, a mimetic, a corresponding isolated, enriched, or purified protein, or a homologous product.

85. The method of claim 83, wherein said dnaN or ORF25 product is indirectly labeled.

86. The method of claim 81, wherein said detecting comprises measurement by phage display.

25 87. The method of claim 81, wherein said detecting comprises measurement by surface plasmon resonance.

88. The method of claim 81, wherein said detecting comprises measurement by Fluorescence Resonance Energy Transfer.

89. The method of claim 81, wherein said detecting comprises measurement of fluorescence polarization changes.
90. The method of claim 81, wherein said detecting comprises a scintillation proximity assay.
91. The method of claim 81, wherein said detecting comprises a biosensor assay.
92. The method of claim 81, wherein said test compound is a small molecule, a peptidomimetic compound, or a fragment or derivative of a bacteriophage inhibitor protein.
93. The method of claim 91, wherein said bacteriophage inhibitor protein is from *S. aureus* bacteriophage AHJD 12 or 25.
94. The method of claim 81, wherein said test compound is a peptide.
95. The method of claim 94, wherein said peptide is an artificially synthesized peptide.
96. The method of claim 94, wherein said peptide is a peptide prepared in expression systems.
97. A method for inhibiting an *S. aureus* dnaN product, comprising contacting said dnaN product with a bacteriophage 44AHJD ORF25 product or fragment thereof at a concentration sufficient to inhibit said dnaN product.
98. The method of claim 97, wherein said contacting is *in vitro*.
99. The method of claim 97, wherein said contacting is in a cell.
100. The method of claim 97, wherein said contacting is *in vivo*.

101. The method of claim 97, wherein said contacting is in a mammal.
102. A method for inhibiting an *S. aureus* dnaN product, comprising
5 contacting said dnaN product with a structural mimetic of a bacteriophage 44AHJD
ORF25 product or biologically active fragment at a concentration sufficient to inhibit said
dnaN product.
103. The method of claim 102, wherein said contacting is *in vitro*.
104. The method of claim 102, wherein said contacting is in a cell.
105. The method of claim 102, wherein said contacting is *in vivo*.
106. The method of claim 102, wherein said contacting is in a mammal.
107. A pharmaceutical composition comprising
a pharmaceutically effective amount of a bacteriophage 44AHJD product or
fragment thereof, and a pharmaceutically acceptable carrier.
108. A pharmaceutical composition comprising
a pharmaceutically effective amount of a structural mimetic of a bacteriophage
44AHJD product of fragment thereof, and a pharmaceutically acceptable carrier.
109. The pharmaceutical composition of claim 120, wherein said structural mimetic is a
peptidomimetic.
110. The pharmaceutical composition of claim 120, wherein said structural mimetic is a
synthetic mimetic.